The Bulletin
of the
American Society of Clinical
Laboratory Technicians

VOLUME 1 MARCH, 1935 NUMBER 2

TECHNIC OF THE KOLMER COMPLEMENT FIXATION TEST FOR SYPHILIS

By CAROLA E. RICHTER

From the Department of Bacteriology and Immunology of Temple University Hospital and the Research Institute of Cutaneous Medicine, Philadelphia, Pa.

Only three changes in the technic of the Kolmer complement fixation test for syphilis have been made since originally described twelve years ago (1), namely:

1. The routine practice of removing natural antisheep hemolysin from all sera which increases the sensitiveness of the reaction although not absolutely required. While not recommended originally this change has been in use over eight years and has been included in subsequent descriptions of the technic for preparing serum for the Kolmer test (2, 3) and need not be here described.

2. The use of a new C. L. antigen re-enforced with acetone insoluble lipoids (4, 5) in dose of 20 instead of 10 antigenic units which has increased the sensitiveness of the test with no increase of non specific reactions.

3. The recent adoption of 0.2 c.c. of serum for the first dose as suggested by Boerner and Lukens (6). This has been found to likewise increase the sensitiveness of the test with practically no increase of anticomplementary or non specific reactions.

New Antigen

This is now prepared in the following manner:

1. Twenty-five grams of beef heart powder (Difco Laboratories) are extracted for five days at room temperature with 200 cc. of ether and the ether saved.

2. The residue is extracted with 200 cc. of ethyl alcohol in an incubator for four days and the alcohol saved.

3. The latter is evaporated and the residue extracted with 30 to 50 cc. of ether. This ether is mixed with the ether of the primary extraction, concentrated and treated with six volumes of acetone to precipitate the acetone-insoluble lipoids (lecithins).

4. After extracting the tissue residue a second time with 100 cc. of absolute ethyl alcohol in an incubator for six days these lipoids, along with 2.0 grams of additional lipoids recovered from the ether extractions of the Kahn antigen and 0.2 gram of cholesterol are dissolved in 20 cc. of ether and added to the secondary alcoholic extract.

5. The mixture is shaken well and placed in a water bath at 55° C. for one hour to aid solution of the lipoids. After allowing to stand at room temperature for several days with daily shaking, the extract is filtered through fat-free paper to remove the undissolved lipoids, measured and sufficient absolute ethyl alcohol (acetone free) added to bring the total volume to 100 cc.

6. The resulting clear extract is titrated in the usual manner (2, 3) except that the dilutions in the antigenic titration are carried up to about 1:10,000, since the extract has increased antigenic activity with slight or no increase of anti-complementary activity.

7. Under the conditions *it is used in dose of 20 units* instead of 10 units for conducting the Kolmer test which increases the sensitiveness of the test without increasing the danger of falsely positive or non-specific reactions.

The antigen should be kept at room temperature where its properties remain unchanged for a year or more when tightly stoppered to prevent evaporation.

Dr. Kolmer has also found that the preparation of this antigen can be simplified by the following alternative method and either may be used:

1. Place 30 grams of beef heart powder (Difco Laboratories) in a flask with 100 cc. of chemically pure acetone. Stopper tightly; shake thoroughly and keep at room temperature for five days with brief shaking each day.

2. Filter and discard the acetone.

3. Dry the residue and extract with 100 c.c. of chemically pure absolute ethyl alcohol in a tightly stoppered flask for five days at room temperature, shaking briefly each day.

4. Filter through fat-free paper with slight squeezing of the tissue.

5. Dissolve 0.2 gram of cholesterol and 2 grams of acetone-insoluble lipoids in 20 cc. of ether and add to the filtrate. Shake thoroughly and place in a water bath at 55° C. for one hour to aid solution of the lipoids.

6. Allow to stand at room temperature for two or three days with brief shaking each day. Filter through fat-free paper. Measure and add absolute ethyl alcohol if necessary to bring the total volume to 100 cc.

7. Titrate in the same manner as originally described (2, 3) except that the dilutions are carried up to about 1:10,000.

8. The extract should be kept tightly stoppered at room temperature where its properties remain unchanged for a year or longer.

9. Both extracts are uniformly free of hemolytic activity in dose of 0.5 cc. of 1:4 dilution; the anticomplementary units are usually 0.5 cc. of 1:6 or 1:8 while the antigenic units are usually 0.5 cc. of 1:6000 or higher. Under the circumstances they are used in dose of 20 instead of 10 antigenic units since this dose is at least 40 to 60 times less than the anticomplementary unit and giving, therefore, a very wide range of safety:

Hemolytic	: absent in 0.5 cc. of 1:4
Anticomplementary	: 0.5 cc. of 1:6
Antigenic	: 0.5 cc. of 1:6000 or higher
Dose	: 0.5 cc. of 1:300 or higher

Change in the Amounts of Serum Employed in the Quantitative Test

Since 1922 the amounts of serum employed in the *quantitative* test have been 0.1, 0.05, 0.025, 0.005 and 0.0025 cc. with 0.1 cc. in the serum control. Dr. Kolmer knew then that the great majority of syphilitic sera gave positive reactions in only the first three tubes but an occasional serum with exceptionally large amounts of antibody reacted in the fourth and even the fifth tubes and his purpose was to use such a range of doses that the fifth tube carrying the smallest amount of serum would give less than a ++++ reaction in order to secure a truly quantitative reaction in the five amounts of serum employed.

But the main objection to these amounts has been the jump from 0.025 cc. in the third to 0.005 cc. in the fourth tubes. Experience with many thousands of tests has shown that syphilic sera may give ++++ reactions in the first three tubes with completely negative reactions in the fourth and fifth tubes because the fourth tube carried only one-fifth of the amount of serum in the third.

In a series of 9530 sera tested during the past two years at Temple University Hospital by Dr. Kolmer and myself, 859 or about 9 per cent gave positive reactions but only 61 of these positive sera showed complement fixation in the fouth and but 42 in the fifth tubes. Dr. Fred Boerner and Miss Lukens (6) have had a similar experience at the Graduate Hospital in that only about 10 per cent of syphilitic sera showed complement fixation in the fourth or fifth tubes or both. Indeed it would appear that not more than 5 per cent of syphilitic sera yield positive reactions in the fifth tube of 0.0025 cc. and ++++ reactions in this dose are of exceptional occurrence.

Dr. Boerner and Miss Lukens have suggested making the first dose 0.2 cc. instead of 0.1 cc. since they have found that this increases the sensitiveness of the test with practically no increase of anticomplementary or non-specific reactions.

Since they brought this fact to our attention about a year ago, Dr. Kolmer and I have conducted 2393 tests with a first dose 0.2 cc. and 0.2 cc. in the sixth or serum control tube. Our results have been in complete confirmation of those observed by Dr. Boerner and Miss Lukens in that there has been a distinct increase in the sensitiveness of the test with no appreciable increase of anticomplementary or non-specific reactions.

For example, in a series of 2393 comparative tests the use of 0.2 cc. of serum in the first tube yielded 4.1 per cent more positive reactions than in tests employing 0.1 cc. as originally described.

Most of these occurred with the sera of treated cases of syphilis and in all but one instance occurred in cases regarded clinically as syphilitic.

Under the conditions Dr. Kolmer has adopted the suggestion of Dr. Boerner and Miss Lukens to use the 0.2 cc. dose of serum and in the *quantitative test* the following amounts are now employed (7):

0.2 cc.
0.1 cc.
0.05 cc.
0.025 cc.
0.005 cc.
0.2 cc. (serum control)

It will be noted that the fifth tube carries but one-fifth the amount of serum in the fourth tube. This is necessary in order to preserve the quantitative feature of the test. Experience has shown that if the fifth dose is one-half the fourth or 0.0125 cc. that anywhere from 10.3 to as high as 30 per cent of syphilitic sera will give ++++ reactions in all five tubes and his object is to use a fifth dose giving less than a ++++ reaction. By using 0.005 cc. in the fifth tube experience has shown that not more than about 7.1 per cent give positive reactions of + to +++ while ++++ reactions are of exceptional occurrence and occurring only with sera carrying unusually large amounts of antibody.

This new range of serum doses is readily and easily set up in the following manner:

1. For each serum arrange 6 test tubes and place the following amounts of saline solution: 0.9, 0.5, 0.5, 0.5, 2.0 and 0.5 cc.

2. To tube No. 1 add 0.6 cc. of hemolysin-free and inactivated serum. Mix by drawing up in the pipet several times and transfer 0.5 cc. to No. 2 and 0.5 cc. to No. 6 (serum control).

3. Mix No. 2 and transfer 0.5 cc. to No. 3.

4. Mix No. 3 and transfer 0.5 cc. to No. 4.

5. Mix No. 4 and transfer 0.5 cc. to No. 5; mix and discard 2.0 cc.

This leaves 0.5 cc. in each of the first 5 tubes carrying the above amounts of serum (0.2, 0.1, 0.05, 0.025 and 0.005 cc.); No. 6 (the serum control) carries 1.0 cc. or 0.2 cc. of serum since it receives no antigen and thereby making the total volume in all tubes the same when the test is finished.

6. The balance of the test and the method of recording and reporting the reactions are exactly as originally described (2, 3) except that the new antigen is used in dose of 20 instead of 10 antigenic units as previously stated.

Change in the Amounts of Serum Employed in the Qualitative Test

In the original test the amounts of serum employed were 0.1 and 0.05 with 0.1 cc. in the serum control. The 0.05 cc. dose was employed because it was very occasionally observed that 0.1 cc. may give a weaker reaction than 0.05 cc. probably because of a pre-zone reaction.

Dr. Boerner and Miss Lukens have proposed a four tube test using 0.2, 0.1, 0.05 and 0.2 cc. (serum control) but we have found that 0.2 and 0.1 cc. with 0.2 cc. in the serum control are sufficient when the new antigen is employed.

This makes a three tube test which is sufficient and very satisfactory for diagnostic purposes. It is not, however, a quantitative procedure and for this reason Dr. Kolmer still prefers to designate it as a qualitative test. It is of course much more quickly set up and more economical and just as good as the quantitative test for diagnostic purposes insofar as positive or negative reactions are concerned but we prefer the quantitative procedure with the sera of all treated cases of syphilis because it gives much better evidence of serologic improvement. It is set up as follows:

1. For each serum arrange 3 test tubes and place the following amounts of saline solution: 0.9, 0.5 and 0.5 cc.

2. To No. 1 add 0.6 cc. of hemolysin-free and inactivated serum. Mix by drawing up in the pipet several times and transfer 0.5 cc. to No. 2 and 0.5 cc. to No. 6 (serum control).

3. Mix No. 2 and discard 0.5 cc.

This leaves 0.5 cc. in each of the first 2 tubes carrying 0.2 and 0.1 cc. of serum respectively. No. 6 contains 1.0 cc. (0.2 cc. of serum) since it receives no antigen and thereby making the total volume in all tubes the same when the test is finished.

4. The balance of the test and the method of recording and reporting the reactions are exactly as originally described (3) except that the new antigen is used in dose of 20 instead of 10 antigenic units.

The amounts of cerebrospinal fluid employed are the same as originally advised (0.5, 0.25, 0.125, 0.0625 and 0.03 with 0.5 cc. in the control); some have advised using a first dose of 1.0 cc. but so far Dr. Kolmer has not been convinced that this is advisable.

Conclusions

1. The C. L. Antigen for the Kolmer test has been improved by reinforcing with acetone insoluble lipoids. It is used in dose of 20 instead of 10 antigenic units which increases the sensitiveness of the reactions while preserving specificity and freedom from falsely positive reactions when the test is otherwise conducted exactly as originally described.

2. The amounts of serum employed in the Kolmer *quantitative* complement fixation test for syphilis have been changed to 0.2, 0.1, 0.05, 0.025 and 0.005 cc. with 0.2 cc. in the serum control.

3. In the *qualitative* test the amounts have been changed to 0.2 and 0.1 cc. with 0.2 cc. in the serum control.

4. By using a first dose of 0.2 cc. of serum the sensitiveness of the reactions has been increased without any increase of non-specific reactions.

REFERENCES

1. Kolmer, J. A.: A new complement fixation test for syphilis based upon the results of studies in the standardization of technic. Am. Jour. Syph., 1922, 6, 1.
2. Kolmer, J. A.: Serum Diagnosis by Complement Fixation. Lea and Febiger, 1928, page 388.
3. Kolmer, J. A., and Boerner, F.: Approved Laboratory Technic, D. Appleton and Company, 1931, page 458.
4. Kolmer, J. A., and Richter, C. E.: A note on the acetone insoluable lipoids in relation to antigen for the Wassermann reaction. Am. Jour. Clin. Path., 1934, 4, 235.
5. Kolmer, J. A.: New antigens for the Kolmer modification of the Wassermann test. Am. Jour. Clin. Path., 1935, 5, 55.
6. Boerner, F., and Lukens, M.: Some modifications of the Kolmer-Wassermann test. Am. Jour. Syphilis (in press).
7. Kolmer, J. A.: Changes in the technic of the Kolmer-Wassermann test. Am. Jour. Syphilis (in press).

A SERUM MEDIUM

By VIVIAN HERRICK, L.T.

Laboratory Division, Hillman Hospital, Birmingham, Ala.

The following method of preparing a coagulated serum medium has been used for nearly two years in lieu of Loeffler's Serum and has proven highly satisfactory. If care is taken a perfect medium is obtained without the use of the inspissator.

The serum left over from serological tests is pooled and if sufficient is not obtained it is stored in the mechanical refrigerator until enough is collected to make up a number of tubes. If it is kept at a low temperature there will be no bacterial growth even if it is kept for several weeks. Serum from blood obtained at the abbatoir may be used but in a large hospital it will be found more convenient to save human serum from the various tests.

Place serum in test tubes of the desired size. Place in a slanting position in a wire basket. Do not put more than two layers in a single basket. The tubes may be plugged with cotton. However, if a basket or can of cork stoppers are sterilized along with the medium and later put into the tubes with a sterile forcep it lessens drying of the medium.

Coagulation: Place in the autoclave and close the door and all of the ports and valves. Open the steam inlet as wide as possible and run the pressure of the chamber to 15 pounds as quickly as possible. Keep a steady pressure of 15 pounds or a little less but *not* more, for 10 minutes. If this pressure is not possible with your autoclave 10 pounds for 20 minutes or even 5 pounds for one hour will suffice. The higher pressure is desirable but not essential.

Sterilization: After the serum is coagulated open the outlet valve and allow enough steam to escape so that the air in the chamber is replaced by steam. While allowing the steam to escape do *not* close the steam inlet as the pressure in the chamber must be maintained during the exchange to prevent boiling as the heat is increased. Keep the pressure of the chamber at 13 to 15 pounds but not more for 20 minutes, or at 10 pounds for 30 minutes or at 5 pounds for 1½ hours. After the time is up cut off the steam inlet and open the safety valve and empty the chamber as rapidly as possible of the steam. Open the door as quickly as possible. The tubes will not blow and bubbling will be avoided. If the pressure is run down too slowly the temperature will be high enough to cause boiling as the pressure approaches the atmospheric and the surfaces will be spoiled.

THE USE OF DILUTE HCl AS A DILUENT FOR COUNTING LEUCOCYTES

FRED BOERNER, V.M.D.*

Walker, (1) 1932, reported the use of N/10 HCl for counting leucocytes after infusion of solution of acacia. He did not, however, recommend its use routinely. We have since found that the use of dilute HCl as a diluent for leucocyte counts in routine work is just as suitable as a solution of acetic acid and of distinct advantage when one of the acid hematin methods (especially the Haden method) is used for the hemoglobin determination. The following experiments are offered in support of the above.

Duplicate counts were made on 25 patients using N/10 HCl as a diluent for one, and 3% acetic acid for the other. The difference between the counts in individual cases were as close as would be expected if two counts were made with the same solution. The total number of leucocytes counted in each series were very close, namely: 3% acetic acid, 168,000 and N/10 HCl, 175,000. This indicates that the HCl does not destroy any of the cells or prevent their detection. These results are in accord with those of Walker who found no significant difference between counts made with N/10 HCl and 0.5% acetic acid. The cells in the N/10 HCl appeared to be a trifle smaller. This difference was so slight that only by careful comparison was it noticeable.

Twenty different concentrations of HCl, ranging from N/1 to N/20 were tested. The results of this study indicated that a range of N/8 to N/12 was most satisfactory. Stronger concentrations failed to completely destroy the red blood cells. When N/1 was used, there was no destruction of the red blood cells. The weaker concentrations acted more like distilled water, in that the red cells were hemolized, leaving ghost or shadow cells and the leucocytes were not distinct. From the above observations it is safe to substitute a 1% solution instead of N/10 whenever it is desirable (or convenient).

The advantage of using N/10 HCl as a diluent for the leucocyte count in laboratories employing hemoglobin methods which require the same solution is obvious. In the first place only one solution is necessary for the two tests; secondly, if the Haden

* From the Laboratories of the Graduate Hospital, University of Pennsylvania, Philadelphia, Pa.

method for hemoglobin determination is used, one pipet may be used for both hemoglobin and leucocyte count. Many of the pipets will not hold enough fluid for both tests. Most of them hold sufficient fluid for a leucocyte count and a determination of hemoglobin when the clinical model of the Haden-Hauser instrument is used.

We consulted Mr. S. Trenner of the Arthur H. Thomas & Co. regarding larger pipets and were informed that a few months before, Drs. Pons and Belks, (2) had made similar inquiries. They, too, had found that N/10 HCl could be used for both leucocyte counts and hemoglobin estimations. They have since been using the Reeder pipet for both determinations with very satisfactory results. For the past seven months we have used N/10 HCl routinely for counting leucocytes and it has proven to be in every way as satisfactory as acetic acid.

Conclusions

1. Dilute hydrochloric acid in a range from N/8 to N/12 is a satisfactory diluent for enumerating leucocytes.

2. It has the advantage in that but one solution is needed for both the leucocyte counts and hemoglobin estimation when acid-hematin methods are used.

3. If the method Haden is used for estimating hemoglobin, only one white cell pipet is necessary for both the leucocyte count and hemoglobin determination.

REFERENCES

1. Walker, M. A.: Tenth-Normal Hydrochloric Acid as a Diluent for Counting Leucocytes after Infusion of Solution of Acacia. J. Clin. Path., 2: 347-349, 1932.
2. Pons, C. A. and Belks, W. P.: Tenth-Normal Hydrochloric Acid as a Diluting Fluid for combined Leucocyte and Hemoglobin determinations. J. Clin. and Lab. Med. in Press.

PIONEERS IN MEDICAL TECHNOLOGY

In the following issues of *The Bulletin* an attempt will be made to publish brief historical sketches with photographic reproductions of some of the prominent historical figures who have had an important influence on medical technology. It is hoped that such a series of brief outlines will be of interest to the reader of *The Bulletin.*

Antony Van Leeuwenhoek

Leeuwenhoek (pronounced Layven-hoch) was born in the Dutch city of Delft on October 24, 1632. At the age of 28 he was appointed to the position of "Chamberlain of the Sheriff" of Delft. This appointment gave him a living income for the support of his family and leisure to carry on his studies in lens grinding.

Leeuwenhoek became an adventurer in the microscopic world, which until his time was practically unexplored. His interest led him to the investigation of microscopic subjects of extraordinary variety, including animals of different kinds and special structures involving the functions of animals, also the structure of plants and vegetative products.

Leeuwenhoek died at the age of 91 years. He has been called the first protozoologist, the first bacteriologist, and may be regarded as the father of applied microscopy.

Editorial

TRAINING OF THE STUDENT TECHNICIAN

The proper training of the student technician has received much thought during the last decade. Certain minimum requirements are becoming recognized and many training schools which meet these requirements have been established.

Dr. Kano Ikeda of the Board of Registry has devoted earnest effort toward the study and analysis of the technician's training and has submitted a report on "A Survey of Training Courses of Laboratory Technicians in General Hospitals".* This report contains much valuable information and should be read by all interested in this subject. He points out that ten educational institutions of collegiate rank are now offering four-year training courses for medical technicians. This marks a distinctly forward step in professional education. At the present time, as is shown by Dr. Ikeda, it is necessary for many hospitals to conduct courses of instruction and training under the direction of qualified clinical pathologists. In the past the clinical pathologist has borne the responsibility of attempting to provide properly trained laboratory workers, and as Dr. Ikeda has shown much of this training is still carried on in the larger hospital laboratories.

It is now recognized that only those hospitals which conform to certain requirements as to personnel and equipment are able to give proper training to students. The opportunities for the smaller hospital laboratories to procure technical help from students is gradually becoming a thing of the past.

As further progress is made in raising the standard of clinical laboratory technique, in classifying laboratory technicians according to their training, experience, and ability, and in providing adequate training for student technicians, it is hoped that more state universities and other institutions of higher learning will become familiar with the need for this form of professional training.

W. E. K.

* Am. Jour. Clin. Path. Vol. 5, No. 1, Jan. '35, p. 64.

News and Announcements

BOARD OF REGISTRY
OF A. S. C. P.

Examinations: At the last semi-annual examination of applicants for certificates of qualification from the Registry of Technicians, held the last week in October, one hundred eighty-two individuals presented themselves for the tests. Sixty-three clinical pathologists in localities in the United States and Canada conducted the examination, which consisted of a practical and a written part. The answers to the written test are marked by members of the Board of Registry and count 25% in the final average; the practical test 50%, personal and psychological attributes 25%, which are judged by the local examiners, constitute the balance of the appraisal of the applicant's fitness. One hundred and seventy went through the ordeal successfully, while twelve fell short of the passing mark of 70.

The Following Questions Were Used in the Written Examination of Applicants for Registration as Laboratory Technicians

General

1. Discuss the relationship of the clinical laboratory technician to the attending physician, the patient and to the directing clinical pathologist in accordance with the Code of Registry of the American Society of Clinical Pathologists.
2. Name the diagnostic or susceptibility tests employed in the following diseases: (a) Typhoid Fever; (b) Lues; (c) Tuberculosis; (d) Diphtheria; (e) Scarlet Fever.
3. Define: (1) Hydrophobia; (2) Symbiosis; (3) Transfusion; (4) Monilia; (5) Autolysate.
4. Describe by drawing the anatomical relationship of the kidneys, ureters, bladder and urethra.

Clinical Bacteriology

5. Name ten well known micro-organisms which may be met in the clinical laboratory. Indicate the comparative morphology of each by drawing and for each mention one important differential cultural, microscopical, or serological characteristic, and for each one common clinical source.

Hematology

6. Define: (1) Reticulocyte; (2) Granulocytopenia; (3) Poikilocyte; (4) Hemopoiesis; (5) Fibrinogen.

Urinalysis

7. Define: (1) Polyuria; (2) Albuminuria; (3) Glycosuria; (4) Oliguria; (5) Anuria.

Serology

8. Give the details involved in conducting the Kahn Precipitation Test with Serum, or other precipitation or immunologic test for lues.

Analytical Methods

9. Outline the preparation of Standard N/10 Sodium Hydroxide Solution.

Examination of Feces

10. What organism is the cause of amebic dysentery and what is the general procedure involved in demonstrating its presence in feces?

The work of the Registry is now very well known through medical and hospital circles. To still further disseminate its usefulness, exhibits have been held by the Registry at several medical and hospital conventions of national scope. The last one was held at San Francisco, February 17-21, at the meeting of the Western Hospital Association, in conjection with the Western Catholic Hospital Association. Plans are being made for a display before the coming meeting of the American College of Physicians, in Philadelphia, and the American Medical Association, in Atlantic City.

NATIONAL

A meeting of the officers of the A. S. C. L. T. was held in Birmingham, Alabama, January 31, and February 1. Present: Mr. Conlin, Miss McCarty, Mrs. Herrick, Miss Murphy. As a result of the meeting the officers made the following recommendations to the Executive Committee:

That a permanent editorial and secretarial office be established for the society with a permanent editor and business manager in charge.

That the president be granted permission to appoint such an officer for a seven-year period.

That Society funds be invested in permanent equipment for this office, including an addressograph machine. This copy of your Bulletin has been mailed out with the help of the new addressograph, which will in the future insure prompt posting of your Bulletin and complete mailing lists.

After obtaining an expression of opinion from the Executive Committee the president is pleased to announce that Mr. Conlin has accepted the appointment and that our permanent offices are now located at 10439 Outer Drive East, Detroit, Michigan.

In the future all applications for membership, payment of dues, inquiries concerning the Society and the Bulletin should be addressed to the office. Notifications of change of address of members should be sent at once.

Headquarters for our Third Annual Meeting will be Haddon Hall, Atlantic City. The plans of the program, exhibit and entertainment committees sound delightful. Our speakers include many old laboratory textbook friends, such as Dr. Haden, Dr. Van Slyke, Dr. Kilduffe and our own Dr. Hillkowitz. Make your reservations for Atlantic City as soon as possible and make every effort to be there.

Drs. Hillkowitz, King and Ikada are not only serving as advissory editors of the Bulletin but are also acting as an advisory body to serve the Society in a general capacity. The president wishes to thank them not only for the generous amount of their valuable time which they have given the Society so cheerfully, but for the thought, acumen and experience which they have applied to our problems since the inception of the Society.

Word comes from Mrs. Herrick, the secretary of the Society, that they have had a deluge of new applications for membership. We find this news most cheering and hope the flood continues until every registered laboratory technician has joined the ranks. We expect the membership to increase steadily as more technicians become acquainted with the purpose of the organization.

Sister M. Joan of Arc Wilson, chairman of the Program Committee for the coming convention, reports that they have received 500 returns from the questionnaire. It yielded a tremendous amount of information. A report will be made at the convention in New Jersey in June.

STATE

Illinois

The Chicago Society of Clinical Laboratory Technicians held its annual dinner at the Medinah Michigan Avenue Club Saturday evening, January 19th. Dr. M. MacEachern and Dr. Williamson of the American College of Surgeons gave short talks. Dr. J. J. Moore, who has encouraged the organization from its infancy, was the toastmaster and he gave everyone some good laughs as well as some serious thought. Dr. F. O. Tonney of the Chicago Board of

Health and Dr. Vida Latham, a pioneer of women in medicine, also spoke.

The results of the election of officers were announced at the end of the dinner. The officers for the coming year are:

President—Mrs. Alma Tillotson, B.S., L.T.

Vice-President—Miss Philomene Cook, L.T.

Secretary—Miss Gladys Eckfeldt, B.S., L.T.

Treasurer—Miss Louise Westley, L.T.

Board Members—Miss Ivie Christianson, L.T., and Mr. George Kneeland, Ph.B., L.T.

Washington

An Inland Empire Clinical Laboratory Technicians' Club has been organized. The first meeting was held in the doctors' club room of the Medical and Dental building, Spokane, Wash., with 15 charter members present. The club is the first of its kind in the west and members will meet monthly for study and research and to cultivate a high ethical standard among laboratory technicians in accordance with a code of ethics established by the American Society of Clinical Pathologists.

Mrs. Jacqueline Bahrenburg was elected president of the new club; Miss Ruth McCulloch, vice-president; Miss Irene Aitchison, secretary-treasurer.

Meetings will be held the second Thursday of each month in the Medical and Dental club room at 7:00 p. m.

Missouri

Miss Margaret W. Brown of State Hospital No. 1, Fulton, Mo., with the aid of other registered technicians is planning a Missouri Society of Clinical Laboratory Technicians. As a preliminary measure they are sending a letter with a questionnaire to all the registered clinical laboratory technicians of the state to see how they respond to the idea. The first meeting will be held in Kansas City, probably in April.

Ohio

Ohio District No. 1 had its annual business meeting and election of officers at St. Thomas Hospital, Akron, Wednesday, January 16th. The following officers were elected for the year:

President—Miss Mabelle Smith, L.T.

Vice-President—Miss Martha Andes, L.T.

Secretary-Treasurer—Miss Ruth Luke, L.T.

A meeting was held Wednesday, February 20th, at Children's Hospital, Akron. Dr. B. S. Kline demonstrated the Kline test and

Dr. H. E. Blass gave a paper on "Some Facts About Syphilis."

The technicians of Ohio District No. 4 are holding their monthly meetings at the Institute of Pathology, Western Reserve University. They are fortunate in securing a definite meeting place right in the heart of the Medical Center and have been able to obtain some of the best pathologists and bio-chemists connected with the University to give them talks and demonstrations connected with clinical laboratory work. As high as sixty-eight technicians have attended one meeting. A more detailed account of their activities is expected later.

Maryland

The Registered Technicians of Maryland had their first meeting in the parlors of Mercy Hospital, Baltimore, April 1st, 1933, and organized under the name of the Maryland Society of Registered Laboratory Technicians. Eight technicians attended the first meeting. Sister M. Joan of Arc, M.T., was elected president and Mr. V. L. Fiannery, M.T., secretary and treasurer. Meetings were later held as follows:

April 29th, 1933, at Mercy Hospital, Baltimore.
Sept. 9th, 1933, at Mercy Hospital, Baltimore.
Nov. 4th, 1933, at Mercy Hospital, Baltimore.
Jan. 6th, 1934, in the Surgical Pathological Laboratory, Johns Hopkins Hospital, Baltimore.
March 3rd, 1934, at Mercy Hospital, Baltimore.
May 5th, 1934, at Mercy Hospital, Baltimore.
Sept. 29th, 1934, at Mercy Hospital, Baltimore.
Jan. 26th, 1935, at Mercy Hospital, Baltimore.

At the January meeting papers were read by Sister M. Joan, Dr. Raymond Peters, Mr. Fiannery, Dr. Eastland, Dr. Schmidt, and a demonstration was made of pneumococcic typing with 32 types sera. Discussions on problems in laboratory technic followed.

The state society now has 15 members and 1 honorary member.

Alabama

The Birmingham Branch of the American Society of Clinical Laboratory Technicians held its monthly meeting on the evening of January 31, 1935. Thirty of the members attended. Miss Sarah McCarty read a paper on "Laboratory Tests for Liver Function," and after an informal dinner the remainder of the evening was given over to the arrangements of topics for discussion at future meetings.

Kentucky

The first annual meeting of the Central Kentucky Society of Clinical Laboratory Technicians was held at the St. Joseph Hospital, Lexington, Ky., on February 4, 1935. The following officers were elected:

President—Miss Ida L. Provost, L.T.

Vice-President—Sr. Mary Antonia Klapheke, L.T.

Secretary-Treasurer—David S. G. Silcock, L.T.

The membership is composed of fourteen registered technicians and the meetings are held on the first Monday of each month.

State and local or district organizations are invited to send in any news of their meetings, elections, or other items of information. Address Sister Alma Le Duc, St. Thomas Hospital, Akron, Ohio. These will be printed in each issue as far as space permits.

Third Annual Convention
American Society Clinical Laboratory Technicians
Atlantic City, New Jersey
June 10, 11, 12, 1935

The Board Walk, Atlantic City, N. J.

Abstracts

TRAINING COURSES FOR LABORATORY TECHNICIANS in general hospitals, a survey, Ikeda, Kano, Am. J. Clin. Path., 5, 64-75, 1935.

This is a report of the information obtained from a questionnaire sent out to 1000 general hospitals. It is to be regretted that only 136 replies were received from hospitals conducting training schools for technicians. The results are tabulated according to bed capacity, geographical distribution and type of hospital (i. e. private, public or university control; approved and not approved).

The results are rather alarming from the technician's point of view. 65.4% of these hospitals training technicians are under 300 beds in capacity, and 16 of the hospitals, while not approved for internship, nevertheless undertake to teach technicians. In such hospitals the clinical material and teaching staff and materials are inadequate to give the technician thorough grounding in all branches of clinical microscopy and laboratory technic. Though the entrance requirments for applicants desiring to enter these training schools have been steadily increased during the past few years, the attention given to their training in a "large number" of hospital laboratories after they enter the hospital is rather haphazard, is conducted strictly on a "practice" basis and follows no organized schedule.

State registration for technicians and the qualifications of laboratory directors are discussed. The author recommends an increase in the ratio of supervising to student technicians, and wherever possible the affiliation of training schools with local universities to combine the didactic and practical training of technicians.

IDENTIFICATION OF PNEUMOCOCCI, the Use of Sodium Desoxycholate for, Leifson, Einar, J.A.M.A., 104, No. 3., P. 213, 1935.

Sodium desoxycholate is recommended for testing the solubility of pneumococci, rather than bile or other bilex salts. The salt is made up in 10% aqueous solution. It is not necessary to sterilize. 2 drops are added per 1 cc of pneumococcus culture. Lysis occurs in 2 to 5 minutes. The pH of the culture must not be below 6.5 and the temperature at which the test is carried out must not be above 50 degrees C.

This salt is far superior to bile and sodium taurocholate, the lysis occuring more uniformly and quickly. None of the strains of streptococci tested by the author have been dissolved. This reagent has been employed for some years abroad, particularly in England but has not been used extensively in this country. The salt can be obtained from Riedel-deHaen, 105 Hudson St., New York.

BLOOD CHOLESTEROL: A modified method for the determination of—Andes, Jerome E., J. Lab. and Clin. Med., 20, number 5, P. 410, 1935.

The author investigated the various drying and extraction methods that can be used in the determination of cholesterol in blood and body fluid with a view to determining the quickest, most practical and accurate method that can be used clinically and carried out by the average technician without previous extensive training in bio-chemistry.

He concludes that fresh plaster of Paris is the most effective substance on which to dry the blood, samples which were undried or dried on filter paper or with hydrated plaster of Paris giving much more variable results. Only 0.25 cc. of blood or serum are required for the determination. Drying for 30 minutes is sufficient, drying for longer periods gives results only slightly higher. Drying at room temperature gives values almost identical as drying at 100° C. and dispenses with the necessity of using an electric oven.

Chloroform is the best extractive, alcohol-ether mixtures give more erratic results and are more expensive. About 30 cc. of chloroform are required.

The extraction and final color development can be carried out in the same tube, thus reducing error caused by loss from transfer. The tube can be made easily and inexpensively from a Folin-Wu Sugar tube.

NEISSERIAN INFECTION, a Differential Stain favorable to the diagnosis of—Scudder, Sara Alicia, Stain Technology, 6, 99-107, July, 1931.

This article, though published some years ago, is abstracted because of its peculiar interest for technicians.

Working with the Color Laboratory of the Department of Agriculture and on cultures supplied by the New York Dept. of Health, Scudder has developed a combination Gram and Pappenheim stain which differentiates the Gonococcus from other organisms com-

monly encountered in urethral, cervical and vaginal smears. The cytology is also far clearer than with the bacteriological stains commonly employed. The method follows:

The exudate is rolled, not smeared, from the swab, dried in air and fixed by gentle heat.

1. Flush the slide with buffered crystal violet, 3 to 5 minutes:
   Crystal violet ..... 1 gm.
   Distilled water ..... 90 cc.
   Buffer solution (pH 6.6 to 7.0) ..... 10 cc.
2. Decant and flush with alkaline iodine solution 1½ to 2 minutes:
   Resublimed iodine ..... 2 gms.
   Distilled water ..... 90 cc.
   N/1 NaOH ..... 10 cc.
3. Decolorize with Merck's pure technical acetone drop by drop until the drippings are colorless. This should not take more than 10 seconds.
4. Counterstain 1½ to 2 minutes in:
   Pyronin yellowish (pyronin G)* ..... 0.1 gm.
   Overethylated methyl green** ..... 1.25 gm.
   Hot distilled water ..... 99 cc.

   * The pyronin yellowish is obtainable from Coleman and Bell.

   ** The overethylated methyl green is obtainable from National Aniline Chemical Co., labelled "methyl green-National."

   The counterstain should be made up a few hours before use.

The stain gives a brilliant red color to the Gonococci. All other Gram negative bacteria stain a faded pink except a small influenza-like bacillus which stains magenta. Gram positive bacteria stain a purplish black. The article is illustrated by a color plate which demonstrates the brilliant results obtained by such a stain.

A modification of Ranson's silver nitrate method for demonstrating axis cylinders. Hewer, Evelyn E.: J. of Anat. 67: 350, 1933.*

A modification of the Ranson method is presented for the purpose of staining axis cylinders.

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* Abstracted from Centralblatt für Allgemeine Pathologie und Pathologische Anatomie 61: January 15, 1935. Translation by S. E. Albert, Feb. 8, 1935.

Book Review

GOULD'S POCKET PRONOUNCING MEDICAL DICTIONARY, Tenth edition, revised. By the late George M. Gould, A.M., M.D. Revised by C. V. Brownlow. P. Blakiston's and Co., Inc., Philadelphia, Publishers. Price: $2.00 Cloth—$2.50 Limp Leather.

A valuable addition to the laboratory book shelf is the tenth edition of this conveniently parcelled compendium of medical and scientific words.

The better to preserve the continuity of the text, Mr. Brownlow has arranged the long lists of definitions which usually follow such "blanket" words as Muscle, Bone, Reflex Bacteria, etc., in condensed tables at the back of the book. The tables on Bacteria and Parasites are compiled by Dr. D. H. Bergey of the University of Pennsylvania. Only the new terminology is given in the bacteriological tables, but under the anatomical tables both the old terminology and the A. S. B. terminology are used and the place in the alphabetical list is preserved by including both names. The table of chemical elements includes other such valuable information as valence, atomic weight and distribution. Tables for the conversion of English into metric systems are also included.

Two dollars or two dollars and a half would be well invested in this volume, since it carries most of the information usually gleaned from the larger and more expensive dictionaries.

AMEBIASIS AND AMEBIC DYSENTERY: by Col. Chas. F. Craig, U.S.A., Professor of Tropical Medicine, School of Medicine, Tulane University of Louisiana. Charles C. Thomas, Springfield (Ill.) and Baltimore Publishers. P. 315. Price $5.00.

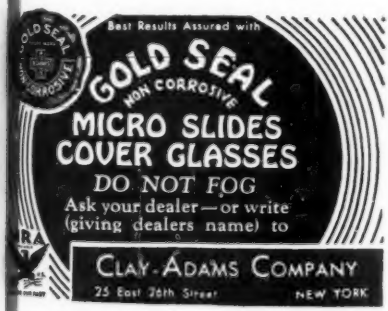
This authoritative monograph should be in the library of any laboratory attempting the diagnosis of amebiasis.

Col. Craig has devoted more than half of the book to discussions

of aspects of the disease which are understandable and significant to any laboratory worker. In particular his reports of surveys by various workers as to incidence and geographical distribution of amebiasis; his chapters on epidemiology including sources and modes of infection, resistance of encysted amebae to the action of physical and chemical disinfecting agents; and on laboratory diagnosis are of great value. The differences in species of commensale and pathogenic amoebae and their life cycles are well explained and are illustrated by numerous and beautiful photomicrographs from the Army Medical Museum collection. Methods for making surveys, collection of specimens, staining, concentrating and culturing are given in adequate detail.

The material is presented in a pleasing and facile style. Discussions of nomenclature and priority of discoveries and publications in this field are courteously and fairly handled. A well indexed bibliography follows.

The book is printed on smooth paper, and is of a comfortable size.



"Sans laboratories les savants sont des soldats sans armes."

—Pasteur

"Men of science without laboratories are as soldiers without arms."

Please Mention Publication When Writing Advertisers

To the Editor

Pasadena, Calif.

I am very pleased to see the first issue of your new Journal and to wish you success in your future endeavors. I think the size of the Journal is just about right. I would not recommend trying to print too large a Journal, particularly since all medical technicians should religiously read the American Journal of Clinical Pathology and the Journal of Laboratory and Clinical Medicine, and those in special fields should read journals relating to their own specialty, such as the Journal of Biological Chemistry for those doing Chemistry. Let me personally congratulate you.

Sincerely,

A. G. Foord, M.D.

Baltimore, Md.

Your letter of Nov. 5th, as well as the first issue of your official publication, is acknowledged. Please accept our congratulations upon your excellent little paper, which certainly should find a ready place in every laboratory. It would seem that you are on the right track to perform a very useful service.

Very truly yours,

La Motte Chemical Products Company,

F. L. La Motte.

Letters should be sent to John H. Conlin. Such letters will be printed in forthcoming issues as far as space permits. Writers should sign their names, which will be omitted on request.

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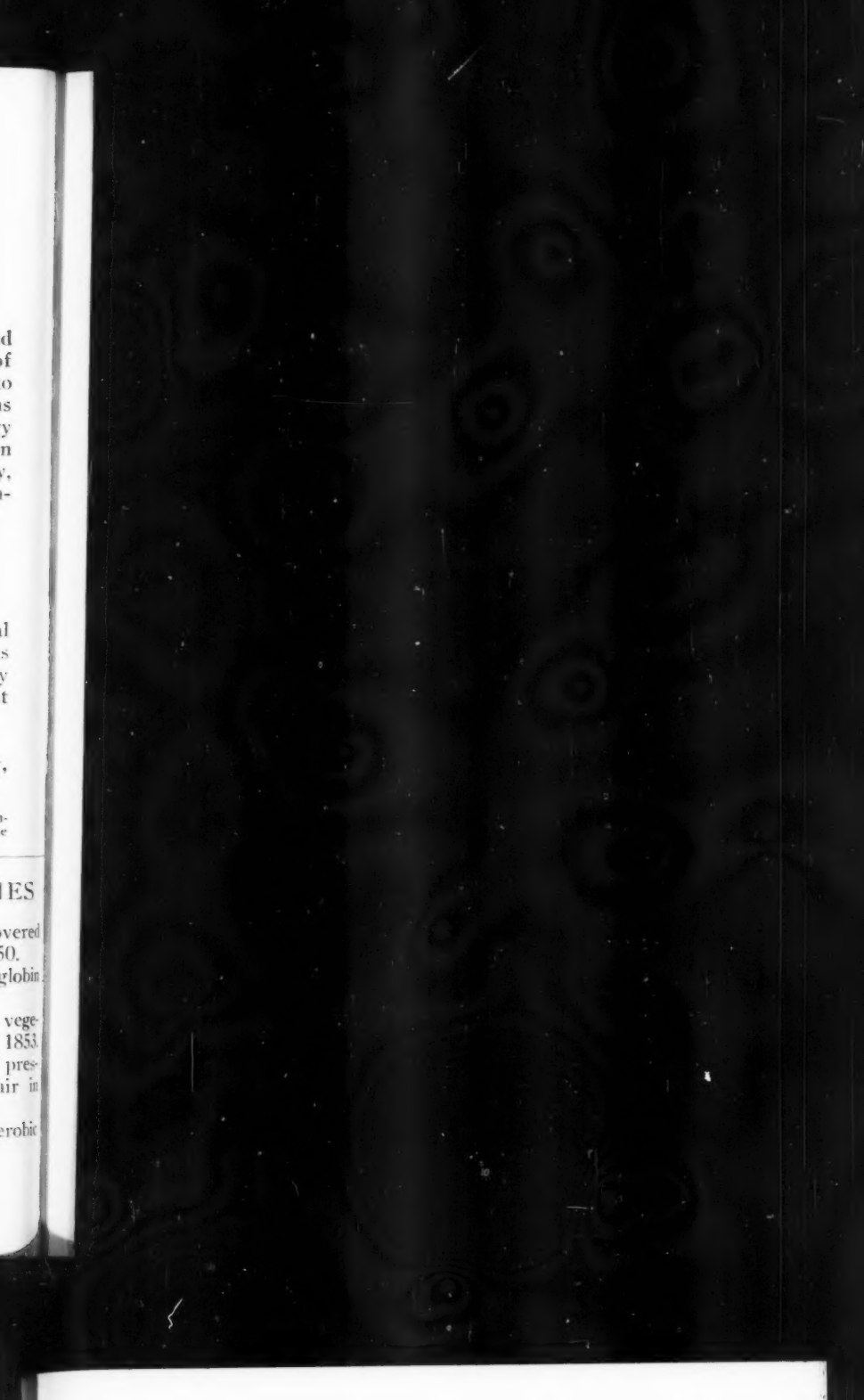
PAST DISCOVERIES

Rayer and Davaine discovered the anthrax bacillus in 1850.

Funke discovered hemoglobin in 1851.

Cohn established the vegetable nature of bacteria in 1853.

Pasteur demonstrated presence of bacteria in the air in 1860.

Pasteur discovered anaerobic bacteria in 1861.